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## Cellulosic ethanol

Sieving methods have been almost exclusively used for feedstock size-reduction characterization in the biomass refining literature. This study demonstrates a methodology to properly characterize specific surface of biomass substrates through two dimensional measurement of each fiber of the substrate using wet imaging technique. The methodology provides more information than sieving methods about biomass substrate. The measured dimensions of individual fibers were used to estimate the substrate external surface based on a cylinder model. The substrate specific surface and mechanical milling energy consumption were then correlated to enzymatic hydrolysis glucose yield. Results indicated that the developed methodology is effective in differentiating various size-reduction and chemical pretreatment processes in terms of cellulose to glucose conversion efficiency and size-reduction energy consumption. Thermomechanical disk milling (DM-I), exposing cellulose, is more effective than a high pressure thermomechanical disk milling (DM-II), exposing lignin, in subsequent enzymatic hydrolysis. However, DM-I is more energy intensive than DM-II. Both DMs that produce fibers are more efficient in enzymatic hydrolysis than hammer milling that produces fiber bundles. Chemical pretreatment not only increased cellulose conversion, but also reduced mechanical milling energy consumption. The present methodology identified the sulfite pretreatment C as the most efficient pretreatment in terms of glucose yield and milling energy consumption.

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With the shortage of petroleum-derived fuel and increased global warming caused by greenhouse gas emissions from combustion of petroleum, there is a renewed interest in developing fuels from biomass. Currently, biofuel in the form of ethanol is mainly produced from starches in food grains. Because of the high production cost of agriculture crops and their limited supply, grain-to-fuel competes directly with food and feed and can be therefore non-sustainable. The next generation of biofuel will be produced from lignocellulosic feedstocks (wood, agricultural residues, and dedicated energy crops) through fermentation of sugars yielded from hydrolysis of two key components of the lignocellulose, the cellulose and hemicellulose.

One of the key barriers to this approach is the physical and chemical resistance of plant cell structure to hydrolysis of cellulose and hemicellulose (Himmel et al., 2007; Sun and Cheng, 2002; Jeon et al., 2007). This resistance is often called recalcitrance, which can be reduced by pretreatment. Extensive understandings of the mechanism of lignocellulose hydrolysis have been developed (Lynd et al., 2002; Mansfield et al., 1999) through years of research. After appropriate level of physical size reduction of biomass that increases the accessibility of the lignocellulose, hemicellulose can be chemically hydrolyzed during a chemical pretreatment step. The hydrolysis of cellulose requires another (subsequent) chemical or enzymatic step after the pretreatment. With the recent advances in enzyme technologies and low environmental impacts, enzymatic hydrolysis is becoming the preferred step for cellulose saccharification.

Woody biomass is a very attractive form of renewable feedstock for liquid biofuel production (cellulosic ethanol) because of its low cost of production, availability in large quantities, and ease in storage. However, major barriers must be overcome for efficient conversion of woody lignocellulose to fuel. Extensive research efforts have been devoted to various pretreatments of biomass to overcome the barriers by partially removing lignin and/or hemicellulose that

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inhibit enzyme accessibility to cellulose. Alkaline, dilute acid, hot water, ammonia, and organosolv pretreatment technologies have been developed with some level of success (Mosier et al., 2005; Gable and Zacchi, 2002; Pan et al., 2005). However, all these pretreatment processes, except the organosolv process, require significant size reduction of the feedstock to fine particles (powders or sawdusts) by mechanical means to achieve satisfactory cellulose conversion (Silverstein et al., 2007; Kim and Lee, 2005; Pan et al., 2005). Unfortunately, significant size reduction is energy intensive. This is particularly true for woody biomass because of the large size and strong integrity of wood. Much research has been carried out on size reduction. However, only energy consumption and substrate size were reported in most size-reduction studies, without conducting cellulose to glucose conversion (Schell and Harwood, 1994; Mani et al., 2004; Womac et al., 2007). Most research work on hydrolysis has used size-reduced substrates without providing information about the energy consumed to produce the substrate and without careful and complete characterization of the substrate (Allen et al., 2001; Zhu et al., 2005; Nguyen et al., 2000). There is a knowledge gap linking energy consumption, substrate surface, and pretreatment efficiency.

In this study, we use fiber fractionation, different chemical pretreatment, and mechanical milling (size-reduction) processes to produce wood substrates with various physical sizes and chemical structures and physical properties. We also apply a wet imaging technique to determine two dimensions of these woody fibrous substrates. The specific surface of a substrate is estimated by using a cylinder model for individual fibers. We conduct enzymatic hydrolysis for each substrate. The determined substrate specific surface is then related to the enzymatic hydrolysis cellulose conversion of the substrate. We also examine the relations of size-reduction energy consumption to specific surface and to enzymatic hydrolysis glucose yield, so that the trade off between size-reduction energy consumption and glucose yield can be demonstrated and the efficiencies of different chemical pretreatments and size-reduction processes can be compared objectively.

## 2. Background

### 2.1. Wood chip size reduction and characterization

There are two steps in wood fiber production. The first step deals with coarse size reduction, i.e., reducing the wood log to a chip size of around 10–50 mm in two dimensions and about 5–10 mm in the third dimension. The second step is to further reduce the wood chip to fibers and/or fiber bundles with lengths of about 2 mm. The energy consumption in the first step is lower than that in the second step. The objective of the second step size reduction is to reduce the heat and mass transfer limitations, which will significantly improve the efficiency of pretreatment and hydrolysis reactions (Tillman et al., 1989, 1990).

Several well developed technologies are available for biomass size reduction, such as hammer milling, knife milling, shredding, and disk or attrition milling. Several studies reported biomass size reduction using these technologies (Holtzapfel et al., 1989; Cadoche and Lopez, 1989; Schell and Harwood, 1994; Mani et al., 2004; Naimi et al., 2006; Womac et al., 2007). Early work on size reduction of woody biomass included those producing wood flour for manufacturing wood composites (Reineke, 1961). The efficiency of a size-reduction process using commercial technologies has been evaluated in terms of energy consumption and the size of the final product, which was often characterized by the geometric mean diameter of the particles (Mani et al., 2004). This was probably because size measurements were often carried out by traditional sieve or screen methods (Dasari and Berson, 2007; Hoque et al., 2007; Cadoche and Lopez, 1989; Schell and Harwood, 1994; Mani et al., 2004). Because

substrate surface area is most relevant to heat and mass transfer and enzyme accessibility, the hydrolysis efficiency combined with substrate specific surfaces should be used in evaluating the efficiency of size reduction. Holtzapfel et al. (1989) used specific surface area to correlate energy consumption for comparing the efficiencies of several size-reduction processes. Their calculation of specific surfaces was based on the assumption that the substrate particles are spherical. Unfortunately, many biomass substrate particles are not spheres but shives or spindles with aspect ratios greater than 10 (Fig. 1). The substrates derived from disk milling processes typically have an aspect ratio of 50 to 100.

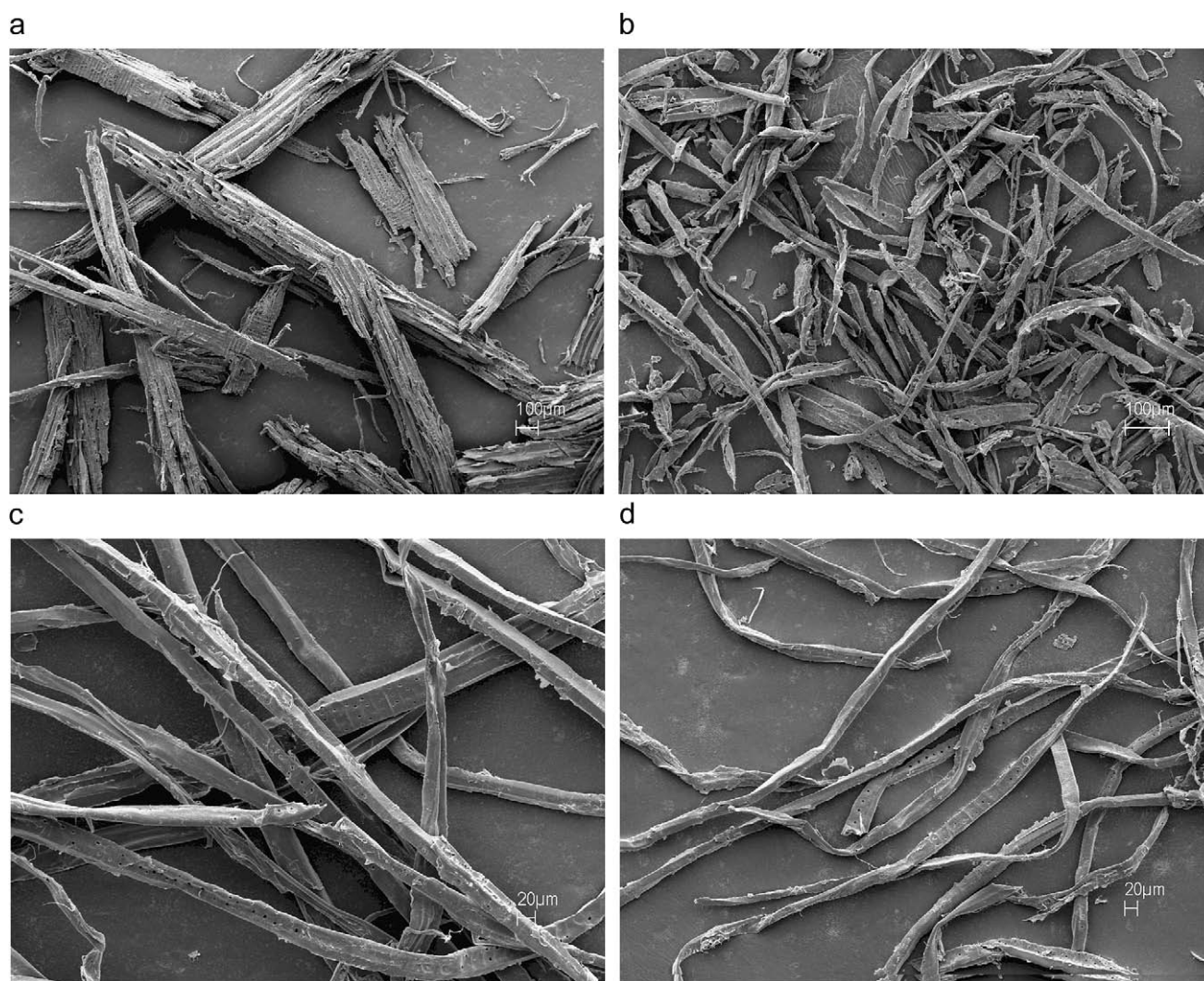
### 2.2. Effect of biomass substrate size on the efficiency of enzymatic hydrolysis

Limited studies have reported the effect of substrate size on cellulose to glucose conversion during enzymatic hydrolysis. Rivers and Emert (1987) concluded that particle size of substrates derived from ball milling had no effect on hydrolysis yield in the wet particle size range of 0.25–0.47 mm studied. Cullis et al. (2004) and Ballesteros et al. (2000) reported that initial wood chip size can affect cellulose saccharification even when the substrates were obtained after steam explosion of the chips. Chundawat et al. (2007) found that particle size affected enzymatic digestibility of ammonia fiber/freeze explosion-(AFEX-) pretreated corn stover. Mooney et al. (1999) indicated that hydrolysis rate is significantly affected by fiber size and fiber surface area. Dasari and Berson (2007) studied the effect of particle size measured by sieving on the glucose conversion using red-oak sawdust collected from a commercial saw mill. They found that glucose conversion rate almost doubled when particle size was reduced from 590–850 to 33–75  $\mu\text{m}$ . Sangseethong et al. (1998) found that substrate hydrolysis efficiency was not affected by the internal pore surface area but rather by the substrate size or external surface (calculated with spherical particle assumption) even though the internal pore surface contributed to over 90% of the total surface of their microcrystalline cellulose. They suggested that the enzyme-accessible internal pore surface depends on the pore depth dictated by the substrate size. As a result, they concluded that particle size is an important structural property of substrate that affects enzymatic saccharification.

Biomass substrates often have a very large and a wide range of aspect ratio (5 to 200). Substrate size measured using traditional sieving and screening methods is significantly affected by the substrate morphology such as aspect ratio. Unfortunately, the sieving method has almost been used exclusively in the biomass refining community for substrate-size characterization, which may limit scientific progress on determining effective size-reduction and chemical pretreatment processes for biomass refining. It is unclear how much internal pore surface contributes to total enzyme-accessible area and what the critical pore depth is from the work of Sangseethong et al. (1998). As a first step, this study will focus on a careful characterization of biomass substrate size and the external specific surface.

## 3. Experimental

The major dependent variables are glucose yield and energy consumption in size reduction. The major independent variables are milling process, pretreatment process, and fiber fractionation. Table 1 lists the experimental design and all the substrates produced through this design. The milling processes are labeled as HM or DM (Appendix A). The chemical pretreatment processes are labeled as A, B, C (discussed later in the text). The fractionated substrates are represented by Rxx (retained) or Pxx (pass) with xx being the mesh size.



**Fig. 1.** Scanning electron microscope (SEM) images of different fractions of hammer-milled (a) HM-R80 and (b) HM-P80 and disk-milled (c) DM-I-R28 and (d) DM-I-R48 substrates.

**Table 1**  
List of experimental conditions and the substrates produced

Milling process	Milling conditions <i>T</i> , <i>P</i> , disk plate gap (mm)	Milling energy (Wh/kg)	Chemical pretreatment	Fractionation	Substrates
Hammer milling	Atmospheric		No	Yes	HM-R20, HM-R40, HM-R80, HM-P80
Disk milling-I	134 °C, 2.4 bar, 0.25, chip pre-steamed	460	No	No	DM-I
				Yes	DM-I-R14, DM-I-R28, DM-I-R48, DM-I-R100, DM-I-R100, DM-I-R200, DM-I-P200
Disk milling-II	166 °C, 7.2 bar, 0.06	151	No	No	DM-II
				Yes	DM-II-R14, DM-II-R28, DM-II-R48, DM-II-R100+R200
		106	A on wood chips then milling	No	DM-II-A
			A on pulp after milling of chips	Yes	DM-II-A-R14, DM-II-A-R28, DM-II-A-R48, DM-II-A-R100, DM-II-A-R200
Disk milling-III1	25 °C, 1 bar, 1.02	124	B on wood chips then milling	No	DM-III1-B
Disk milling-III2	25 °C, 1 bar, 0.76	170	B on wood chips then milling	No	DM-III2-B
Disk milling-III3	25 °C, 1 bar, 1.02	362	B on wood chips then milling	No	DM-III3-B
Disk milling-III	25 °C, 1 bar, 0.76	19	C on wood chips then milling	No	DM-III-C

### 3.1. Materials

Fresh spruce chips were donated by the Wisconsin Rapids mill of Stora Enso, North America (now New Page Corporation,

Dayton, OH). The chips were kept frozen at a temperature of about  $-16^{\circ}\text{C}$  before use. As a standard practice in wood fiber production, the chips were screened using two sieves of mesh size 22 and 8 mm to remove very large and very small chips. This screening is not

necessary for producing substrates for enzymatic hydrolysis research. However, it helped to maintain a constant chip feeding rate in disk milling. Celluclast 1.5 L (cellulase) and Novozym 188 ( $\beta$ -glucosidase) were used as received from Sigma-Aldrich (St. Louis, MO) for enzymatic hydrolysis.

### 3.2. Mechanical size reduction

Mechanical size reduction was conducted using a laboratory 28-cm hammer mill (Montgomery-Ward Model WB9A, 5 HP, 3600 rpm) and a 30.5-cm disk refiner (Andritz Sprout-Bauer Pressurized Refiner, Andritz Sprout, Muncy, PA) at the US Forest Service, Forest Products Laboratory, Madison, Wisconsin. Three types of disk milling were conducted, DM-I, DM-II, and DM-III. The electric energy consumption in disk milling was recorded. The standard deviation was less than 5% based on duplicate experiments. Detailed description of the size-reduction experiments and the DM-I, DM-II, and DM-III milling processes can be found in Appendix A.

### 3.3. Chemical pretreatment

Part of the disk-milled pulp derived at a steam pressure of 7.218 bar (DM-II) was further chemically pretreated (A) using a solution of 7% sodium hydroxide and 12% urea under freezing conditions ( $-18^{\circ}\text{C}$ ) for 24 h (Zhao et al., 2008). The liquid to wood ratio was 6:1. The same pretreatment (A) was also applied to wood chips directly prior to producing the unfractionated DM-II-A substrate by disk milling. A novel process (SPORL) using sulfite to overcome recalcitrance of lignocellulose (Zhu et al., 2008) was applied directly to spruce wood chips. The sodium sulfite and sulfuric acid charges on oven dry wood were 9% and 3.68%, respectively. The liquid to wood chip ratio was 5:1. The pretreatments were conducted at temperature  $180^{\circ}\text{C}$  (B) and  $170^{\circ}\text{C}$  (C) for 30 min in rotating autoclave type pulping digesters. The pretreated wood chips were then disk-milled under atmospheric conditions (DM-III). The three pretreatments are used to demonstrate the application of the present external specific surface methodology to realistic hydrolysis scenarios.

### 3.4. Fiber fractionation

Enzymes generally attack the finest particles of a substrate first. The large particles can remain intact even after many hours of hydrolysis. Fiber flocculation occurred when hydrolyzing long-fraction samples, which further affects the enzymatic digestion of the long fraction due to poor mixing. As a result, the measured cellulose conversion is more representative of the fine particles within the substrate, rather than the whole sample. This obscures the actual size effect on cellulose conversion. Therefore, using samples with relatively uniform size (length or width) is desirable to demonstrate the size effect on cellulose conversion. Unfortunately, in disk milling, one cannot avoid producing samples with a dispersed mass distribution over a wide range of fiber length. In addition, these samples have a relatively constant amount of fines of about 15–25% by mass even though the mean (or bulk) lengths of the samples are significantly different. This occurs independent of the operating conditions, such as disk gap and torque. Fines are commonly referred to fiber lengths  $< 0.02$  mm in the pulp and paper industry. To address this problem, we fractionated disk-milled samples as described in Appendix A to reduce the effect of sample size non-uniformity on cellulose conversion. The fractionation provides substrate samples with very different mean sizes even though produced from the same size-reduction process; therefore, the effect of substrate size on hydrolysis yield can be clearly isolated and the proposed external specific surface methodology can be demonstrated. The drawback of this approach

is that we were not able to obtain the individual size-reduction energy consumption for these fractionated samples.

### 3.5. Two dimensional measurements of substrate using wet imaging

The two dimensional characterization of the substrate length and diameter or “width” for most fiber fractions was conducted using a MorFi LB-01 Fiber Analyzer (Techpap, France). The characterization was courteously provided by Integrated Paper Service (IPS, Appleton, WI) and Techpap (Norcross, GA). The basic principle of the system utilizes optical microscopy and a CCD camera to image fibers in a laminar water flow. The spatial resolution of the image system is  $3\text{ }\mu\text{m}$ , sufficient to resolve most fibers in typical samples with a diameter around  $20\text{ }\mu\text{m}$  or greater (Fig. 1). The low sensitivity to very small particles will produce biases to small specific surface. However, the mass fraction of the very small particles ( $< 3\text{ }\mu\text{m}$ ) is low in most samples, which alleviates the biases. The MorFi analyzer simultaneously measures the length and diameter of each fiber in a water suspension and flowing in a channel. The oven dry weights of the samples to be analyzed were first determined and used for specific surface calculations. Samples from hammer milling (dry) were first soaked in water under ambient conditions for several hours. During testing, the typical fiber mass concentration of the pulp fed to the system was about 0.3–0.5%. The operator observes the images acquired to determine the optimal fiber mass concentration that avoids too many (causing fiber overlapping) or too few (slowing analysis) fibers. The temperature of the flow channel was controlled at  $30^{\circ}\text{C}$  (the allowed range is  $\pm 5^{\circ}\text{C}$  of ambient temperature to avoid fogging of the flow cell glass). Tens of thousand of fibers were measured for each sample to obtain good representation. The system software corrects for fibers cut by the image frame. The MorFi Analyzer has been widely used in the pulp and paper industry for fiber analysis. Guy et al. (2005) compared various wet imaging techniques for measurements of various commercial wood pulps. They found that the relative standard deviations (RSD) in measuring mean length and diameter of a softwood mechanical pulp using a MorFi analyzer were 1.6% and 0.5%, respectively. Assuming that variations in the measured length and width are three times of these respective standard deviations, we estimated the measurement standard deviation in mean volumetric specific surface to be 1.5%.

The hammer-milled fractions, i.e., the HM-R20 and HM-R40, contain many very wide particles that can block the flow cell of the MorFi Analyzer. The substrate length and diameter measurements for these fractions were therefore carried out in-house using traditional optical microscopy. The images were processed by the UTHSCSA ImageTool software (free download at <http://ddsdx.uthscsa.edu/dig/itdesc.html>). The software provides the area and perimeter of each individual object in the image. With the assumption that the image objects are rectangular, the width or diameter of the hammer-milled substrate can be found. Triplicate measurements of the R40 sample indicated that the measurement standard deviation in specific surface was 17.4%. The large standard deviation is mainly due to the limited fibers ( $\sim 150$  vs several thousands in MorFi) imaged with the semi-manual data transferring and processing. All measurements were conducted using wet substrate to simulate enzymatic hydrolysis conditions. The comparability between the MorFi and the in-house microscopy measurements was verified by comparing the measured specific surfaces of the HM-R80 sample from both techniques. The difference was found to be about 15%, within the standard deviation of the in-house microscopy technique.

### 3.6. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated substrates was carried out at 2% of cellulose (w/v) in 50-ml sodium acetate buffer using a



shaker incubator at 200 rpm (MaxQ Mini 4450, Barnstead International, Dubuque, IA). The pH and temperature were adjusted to 4.8 and 50 °C, respectively. A mixture of Celluclast 1.5 L with an activity loading of approximately 30 FPU/g cellulose and Novozym 188 with an activity loading of approximately 45 CBU/g cellulose was used for enzymatic hydrolysis. An excess of Novozym 188 was used to prevent cellobiose accumulation (Emmel et al., 2003). Hydrolysates were sampled periodically for sugar analysis. Each data point was averaged from two replicates.

### 3.7. Analytical methods

Cellulase activity of Celluclast 1.5L was determined by the filter paper method (Wood and Bhat, 1988), expressed as filter paper unit (FPU). Whatmann 1 filter paper was used as a standard substrate. Cellobiase activity of Novozym 188 was determined using *p*-nitrophenyl- $\beta$ -D-glucoside as substrate recommended by the International Union of Pure and Applied Chemistry Biotechnology Commission, expressed as cellobiose unit (CBU).

The chemical contents of the different fractions of spruce feedstock and substrates were measured at the US Forest Service, Forest Products Laboratory by the Analytical Chemistry and Microscopy Laboratory using an improved high performance anion exchange chromatography (Dionex, Sunnyvale, CA) with pulsed amperometric detection (HPAEC-PAD) method (Davis, 1998). Acid hydrolysis was applied to wood chips or substrates to produce hydrolysates for carbohydrate analysis. The measurement RSD of each species were reported (Table 4) based on internal regular quality assurance and quality control. For quick analysis in the enzymatic hydrolysis experiments, only glucose was measured in the resulting hydrolysates using a commercial biochemical analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH). The instrument precision is about 2% based on manufacturer specifications.

## 4. Analysis

### 4.1. Biomass substrate specific surface

Depending on the size-reduction process, the morphology of a biomass substrate can be very different (Fig. 1). In the present study, the particle form is referred to as a biomass substrate with an aspect ratio close to unity; therefore, a sphere approximation of the substrate can be made. In contrast, the fiber form is referred to as a substrate with very large aspect ratios similar to fibers; therefore, the substrate can be approximated as either a cylinder or a ribbon. The rationale for the cylinder assumption is supported by the scanning electron microscope (SEM) images of substrates from disk and hammer millings (Fig. 1).

For the particle substrate, the physical dimension is often represented by its mean diameter. The particle size distribution of a given substrate can be measured by a variety of techniques. The traditional techniques are the sieve and screen methods. Modern techniques include imaging analysis. Imaging techniques measure projection dimensions of a particle. Once the particle size distribution is obtained, there are several statistical ways to calculate the mean particle size. Particles are assumed to be spherical. The arithmetic mean  $D_{10}$ , Sauter mean  $D_{32}$ , and volume (mass) mean  $D_{30}$  diameters can be calculated (Lefebvre, 1989; Sowa, 1992). Neglecting particle surface roughness, the external volumetric specific surface,  $S_p^V$ , can be estimated according to the equation

$$S_p^V = \frac{A_p}{V_p} = 6 \frac{\sum n_i d_i^2}{\sum n_i d_i^3} = \frac{6}{D_{32}} \quad (1)$$

where  $A_p$ ,  $V_p$  are the total surface area and volume of the particles, respectively, and  $n_i$  is the number of particles in size bin  $i$  with

representative diameter  $d_i$ .  $D_{32}$  is also called Sauter mean diameter (SMD).

By measuring the oven dry weight of the sample,  $m_p$ , before analysis, the specific surface,  $S_p$ , of the sample can be determined using the following expression when each fiber (particle) in the sample is accounted for and measured:

$$S_p = \frac{A_p}{m_p} = \frac{\pi \sum n_i d_i^2}{m_p} \quad (2)$$

Most existing size-reduction technologies or processes produce fibrous substrates with very large aspect ratios in a range of about 5–200. Neglecting surface roughness and assuming cylindrical shape, the volumetric specific surface of fibers,  $S_f^V$ , can be estimated according to the equation

$$S_f^V = \frac{A_f}{V_f} = \frac{2 \sum n_i (d_i^2 + 2 \cdot d_i \cdot L_i)}{\sum n_i d_i^2 \cdot L_i} = \frac{4 \sum n_i d_i (L_i + d_i/2)}{\sum n_i L_i \cdot d_i^2} \quad (3)$$

For most fibers with aspect ratios greater than 5, Eq. (3) can be approximated to

$$S_f^V \approx 4 \frac{\sum n_i L_i \cdot d_i}{\sum n_i L_i \cdot d_i^2} = \frac{4}{D_{L21}} \quad (4)$$

where  $A_f$ ,  $V_f$  are the total surface area and volume of the fibers, respectively.  $n_i$  is the number of fibers in fiber group  $i$ .  $L_i$  and  $d_i$  are the representative length and diameter of fiber group  $i$ , respectively. Assuming the sample can be characterized by  $K$  diameter bins and  $J$  length bins, then the total fiber groups  $I = K \times J$ . One can always use two summations with different subscripts,  $k$  and  $j$ , to differentiate diameter bins from length bins in Eqs. (3) and (4).  $D_{L21}$  is a fiber length weighted-surface-length mean fiber diameter or “width.” The difference in volumetric specific surface between Eqs. (3) and (4) is very small, varying from 1% to 5% for the samples used in this study, when calculated using the data measured by the wet imaging method.

Similarly, the specific surface of the sample,  $S_f$ , can be determined by measuring the oven dry weight of the sample,  $m_f$ , before analysis when each fiber (particle) in the sample is accounted for and measured:

$$S_f = \frac{A_f}{m_f} = \frac{\pi \sum n_i (d_i^2 + 2 \cdot d_i \cdot L_i)}{m_f} \approx \pi \frac{\sum n_i \cdot d_i \cdot L_i}{m_f} \quad (5)$$

It should be pointed out that the volumetric specific surface of prolate spheroid-shape fibers with large aspect ratio is very close to the results from Eqs. (4) and (5) based on mathematical analysis.

Fibers have lumens; however, a hollow cylindrical fiber with a very thin wall thickness is a more realistic assumption. If we ignore the wall thickness, then the specific surface is simply two times the specific surface of a cylinder (Eqs. (3) and (5)). Due to the difficulties in determining the internal structural surface of the lumens with existing technology, we simply neglect the lumen surface in this study. This simplification can be reasonable for substrates in single fiber form such as those disk-milled (Figs. 1c and d) and very finely hammer-milled (Fig. 1b), because some parts of a lumen are often collapsed during the size-reduction process, making the lumen surface not easily accessible to enzymes. Furthermore, the pore depth effect suggested by Sangseethong et al. (1998) and Levenspiel (1972) can limit enzyme accessibility into the internal surfaces of pores or lumens. However, for substrates mainly consisting of fiber bundles, such as some hammer-milled samples (Fig. 1a), it is not appropriate to neglect the internal lumen surfaces in determining specific surface because the lumens may not collapse in processing. Unfortunately, it is very difficult to find a simple technique to determine lumen internal surfaces of fiber bundles that can be easily implemented for rapid and routine lab analysis using existing technology.

According to Eqs. (3)–(5), at least two parameters are required to characterize fiber-form substrate. If only one parameter has to be used, Eqs. (1) and (4) clearly indicate that  $SMD$  or  $D_{32}$  should be used to measure the size-reduction efficiency of equipment or processes (such as sawing) that produces spherically shaped particles with aspect ratios close to unity. The length-weighted mean surface-length diameter or width,  $D_{L21}$ , should be used to measure the size-reduction efficiency of equipment or processes, such as disk or hammer milling, that produce cylindrically shaped fibers with large aspect ratios ( $> 5$ ).

Besides the unaccounted lumen internal structural surface, the geometric shape of the cross section of fibers also affects the accuracy of the cylinder specific surface model (Eqs. (3)–(5)). For a given imaging measured projection diameter, the deviation in specific surface from a cylinder is about 40% for fibers with a diamond cross section. The error increases to about 450% for a ribbon fiber with a thickness of  $\frac{1}{10}$  of the measured projection diameter. Fortunately, most fibers produced from disk refining are not all ribbon like (Figs. 1c and d). It is very important to point out that fibers were suspended and flowing in a channel in the wet imaging method (MorFi). Fibers can freely rotate, therefore, the probability of imaging the width and thickness of a ribbon fiber are the same. So, the error in mean specific surface calculated using the cylinder model is significantly reduced on average, when tens of thousands fibers were measured. For example, the specific surfaces of a ribbon fiber (with thickness of  $\frac{1}{10}$  of width) calculated using the cylinder model for two orthogonal orientations are  $4/d$ , and  $40/d$ , respectively, where  $d$  is the width of the fiber. The average of the two is equal to the actual specific surface of the ribbon fiber  $22/d$ . This last example is not intended to deemphasize the errors caused by the geometric shape of fiber cross section, rather to demonstrate that we have taken the best approach currently available (wet imaging) to address a very difficult problem.

#### 4.2. Substrate cellulose conversion

In this study cellulose conversion refers to enzymatic hydrolysis of cellulose to glucose in a substrate, whether chemically pretreated or not. The glucose yield of the enzymatic hydrolysis is obtained from the glucose concentration in the enzymatic hydrolysate. Each glucan unit produces one glucose molecule after gaining one water molecule, therefore the ratio of molecular weight between one glucan unit and glucose of 0.9 ( $C_6H_{10}O_5/C_6H_{12}O_6$ ) is used in the calculation of cellulose conversion:

$$\begin{aligned} \text{cellulose conversion (\%)} \\ = 90 \times \text{glucose yield (weight)} / [(\text{od substrate weight}) \\ \times (\text{substrate glucan content})] \end{aligned} \quad (6)$$

### 5. Results and discussions

#### 5.1. Substrate characterization

We used a pulp sample from disk milling at 2.4 bar and 134 °C (DM-I) and a second sample from hammer milling with very

different morphology (Fig. 1) to illustrate the various cross sectional differences, shapes, and areas of wood fibers. The images shown are fractionated substrates as described in Appendix A. Tables 2 and 3 show the mean values of the various measures of the fractions, including specific surface, derived from these two pulp samples. The data in the two tables indicate that mesh size of each fraction is very different from wet imaging measured mean length and width. Although the mesh size correlates to the mean length, mean width, and even mean specific surface, the correlations for the disk-milled fractions differ significantly from those for the hammer-milled fractions (Fig. 2). In other words, the correlation between mesh size and other size measures of substrates including specific surface depends on the substrates morphology and how the substrates were produced. The disk milling process breaks wood chips mainly into fibers, even for the mesh size 28 and 48 samples (Figs. 1c and d). Hammer milling fiberizes only a small fraction of wood chips in our study. For example, the particles in HM-R80 sample are essentially fiber bundles (Fig. 1a). Only the HM-P80 sample was fiberized (Fig. 1b). Further examining the distribution of various measures of the pulp fractions using MorFi indicates that the fractionated substrates have narrow ranges of length, diameter, and width. The length data agree with that reported by Gooding and Olson (2001). Similar results were obtained for the hammer-milled substrates fractionated by dry sieving.

#### 5.2. Cellulose to glucose conversion

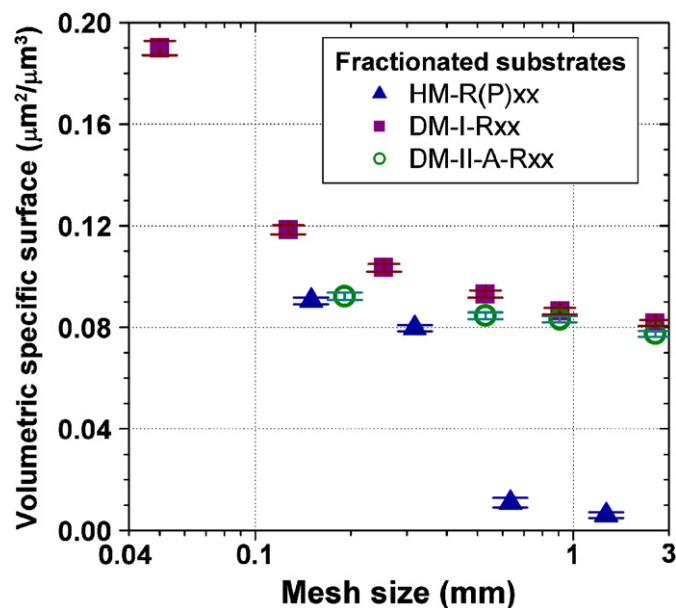
Table 4 shows variations in the chemical compositions of the fractionated and three chemically pretreated substrates from disk milling-I (DM-I). The measurement standard deviations of different species were obtained from regular internal quality assurance and quality control. Lignin content of the fractions was measured using the Klason method, i.e., acid-insoluble lignin (Kirk and Obst, 1988; Lai and Sarkanen, 1971). Wood cellulose content is represented by glucan in Table 4. The rest are hemicelluloses. There is a general trend that glucan is more associated with the coarse fractions while lignin is more associated with the fine fractions. However, the variations are very small except for the DM-I-P200 substrate. The measured glucan content of each substrate was used in cellulose conversion calculations. Fig. 3 shows the effect of substrate mesh size on the time-dependent cellulose conversion percentage for disk-milled and hammer-milled substrates. The standard deviation in cellulose conversion was 2% obtained from duplicate hydrolysis experiments (not shown for clarity). The results clearly show that substrate size affects cellulose conversion. The smaller the mesh size of the substrate, the higher the cellulose conversion. The size effect on cellulose conversion exists even after chemical pretreatment (A) of the substrates. However, when comparing cellulose conversion between the disk-milled (DM-I) and hammer-milled (HM) substrates, smaller mesh size does not correspond to higher cellulose conversion, e.g., HM-R20, HM-R40, HM-R80, have smaller mesh size than DM-I-R14, DM-I-R28, DM-I-R48 but produced significantly lower cellulose conversion than their corresponding disk-milled substrates. As shown in Fig. 2, the specific surfaces of hammer-milled substrates are smaller

**Table 2**  
Different measures of mean size for a set of fractionated disk-milled substrates

Fractions	Mesh size	Arithmetic mean length (mm)	Arithmetic mean diameter (width, $\mu\text{m}$ )	Mean aspect ratio	Mean volumetric specific surface ( $1/\mu\text{m}$ )
DM-I-R14	$> 1.814$ (mm)	1.939	44	44.1	0.0817
DM-I-R28	0.907–1.588	1.817	42.4	42.9	0.0864
DM-I-R48	0.529–0.907	1.286	40.3	31.9	0.0931
DM-I-R100	0.254–0.529	0.723	36.3	19.9	0.1035
DM-I-R200	0.127–0.254	0.403	30.7	13.1	0.1184
DM-I-P200	$< 0.127$	0.269	24.2	11.1	0.19

**Table 3**  
Different measures of mean size for a set of fractionated hammer-milled substrates

Fractions	Mesh size	Arithmetic mean length (mm)	Arithmetic mean diameter (width, $\mu\text{m}$ )	Mean aspect ratio	Mean volumetric specific surface ( $1/\mu\text{m}$ )
HM-R20	> 1.27 (mm)	2.664	230.7	11.6	0.00612
HM-R40	0.635–1.27	1.920	201.5	9.5	0.01092
HM-R80	0.318–0.635	0.531	41.1	12.9	0.07972
HM-P80	< 0.318	0.424	36.6	11.6	0.09044



**Fig. 2.** Correlations of mesh size with substrate specific surface for disk- and hammer-milled substrates.

than the specific surfaces of disk-milled substrates under equivalent mesh sizes. Therefore, comparison of the effect of substrate size on cellulose conversion between the disk and hammer milling cannot be made without considering substrate specific surface.

Fig. 4 shows the cellulose conversion percentages after 48 h of enzymatic hydrolysis as a function of substrate external volumetric specific surface for the untreated substrates. The results clearly show that cellulose conversion percentages increase as substrate external specific surfaces increase for all substrates produced by different size-reduction processes. The results also indicate that DM-I produced higher cellulose conversion percentages than DM-II and hammer milling when comparisons are made under same specific surface. DM-I was a thermomechanical pulping process. More cellulose molecules were exposed on the substrate surface because wood chips were fiberized into individual fibers and fiberization occurred in the S-2 layer with high cellulose content. In DM-II, most wood chips were fiberized into individual fibers and fiberization occurred in the middle lamella (the lignin-rich layer) due to the milling temperature being greater than the glass transition point of lignin; therefore, the substrate was covered with lignin. This can be clearly seen from the color differences between the substrates from DM-I (light) and DM-II (brown). The lignin-covered substrate certainly had lower cellulose conversion efficiency. The data point for the very fine sample DM-I-P200 has completely different chemical composition from those of the rest of the DM-I-Rxx samples (Table 4). The DM-I-P200 contains about 40% more lignin and 33% less glucan than the rest of the DM-I-Rxx samples. Furthermore, this sample has a

much larger surface area so that it does not scale well with the rest of the data in graphs. For clarity in data presentation, we eliminated this sample in Figs. 4, 6, and 7.

Hammer milling produced mainly fiber bundles rather than individual fibers even for the HM-R80 fraction (Fig. 1a). As a result, hammer milling is not an efficient size-reduction process to create surface area for enzymatic hydrolysis. Fig. 4 also shows that the cellulose conversion efficiency of a hammer milling produced substrate HM-R80 is lower than that of a disk-milled substrate DM-I-R48, even though the mesh size of HM-R80 is smaller (0.32 mm) than the mesh size of DM-I-R48 (0.53 mm). This clearly indicates that mesh size is not adequate to compare substrates produced from different size-reduction processes.

Fig. 5 shows the effects of three different chemical pretreatments on cellulose conversion with respect to mean substrate volumetric specific surface. We would like to emphasize the term of mean specific surface in the following discussion. The results indicate that the sulfite pretreatment (B) combined with atmospheric disk milling (DM-IIIx,  $x = 1, 2, 3$  see Table 1) is more effective than the cold alkaline pretreatment A with high temperature milling (DM-II). The DM-III-C substrate has the highest digestibility, with cellulose conversion over 90%. The effectiveness of sulfite pretreatment C can be seen from the large specific mean surface area and low energy consumption in milling to be discussed later.

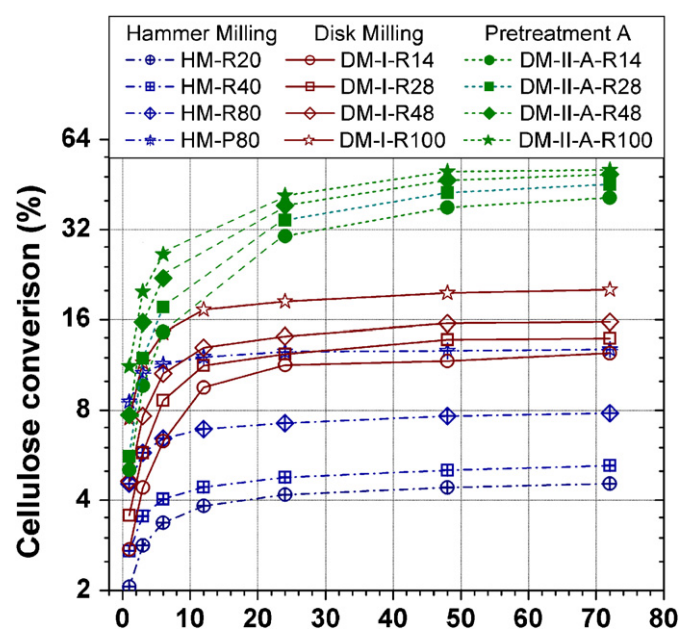
Fig. 5 also shows a smaller slope in cellulose conversion vs mean volumetric specific surface for the unfractionated pretreated substrates (DM-IIIx-B) than the slope of the fractionated substrates (DM-II-A-Rxx). This suggests that increasing mean specific surface by means of size reduction has a significant effect for the fractionated substrates (DM-II-A) comparing to the unfractionated substrates (DM-IIIx-B). This is because the fractionated substrates have relatively uniform fibers, and therefore the mean specific surface is closer to the individual specific surface of the particles. The effect of enzyme accessibility on cellulose conversion can be effectively characterized using the mean specific surface. As discussed previously, the uniformity in fiber specific surface of the unfractionated substrates is poor. These substrates contain some very small fibers that can be easily digested by the enzymes, in particular the substrates were chemically pretreated. They also contain some very large fibers that can remain intact after many hours of enzymatic digestion. When the increase in the mean specific surface is mainly due to the reduced size of the small fibers and not due to increased mass fraction of the small fibers, the increase in cellulose conversion will be limited.

Fig. 6 shows the comparison of cellulose conversion efficiencies between untreated disk-milled and hammer-milled substrates at two different enzymatic hydrolysis duration times. The results indicate that hammer milling produced a higher cellulose conversion percentage than disk milling at the first hour under same volumetric specific surface. In other words, the initial hydrolysis rates of the hammer milling substrates are faster than those for disk milling. This is probably because many very small particles in a hammer milling substrate were attached to the larger particles of the substrate by static force during dry sieving, as visually observed. The very small particles have a very large specific surface and can be

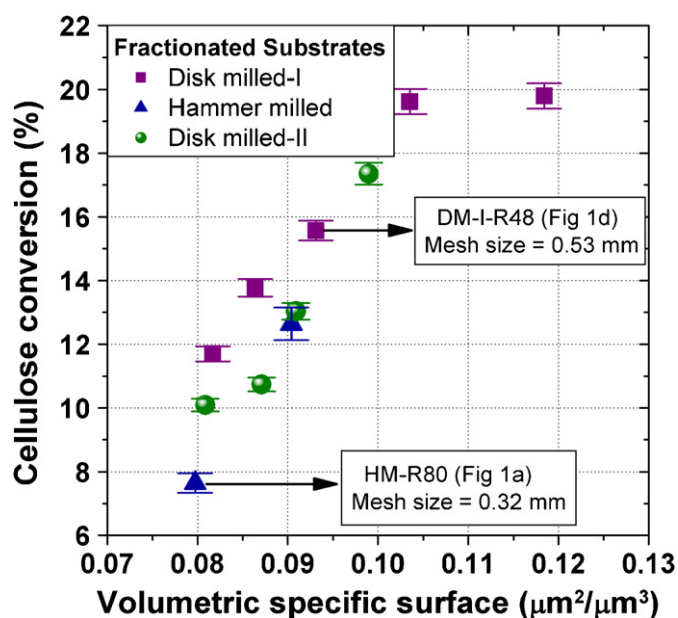
**Table 4**

Chemical compositions of the fractionated substrates from the disk milling-I substrates

	K. Lig. <sup>a</sup> (%)	Arabinan (%)	Galactan (%)	Rhamnan (%)	Glucan (%)	Xylan (%)	Mannan (%)	Sum (%)	Pretreatment solid yield
Spruce wood	28.6	1.24	2.40	0.12	42.8	5.8	11.2	92.1	
DM-I-R14	26.3	0.94	1.66	0.08	46.8	5.7	12.0	93.4	No pretreatment 100%
DM-I-R28	26.1	0.97	1.76	0.08	46.1	5.8	11.9	92.7	
DM-I-R48	27.5	1.01	1.93	0.07	45.1	6.0	11.7	93.4	
DM-I-R100	27.5	1.08	2.23	0.10	44.3	6.1	11.2	92.4	
DM-I-R200	28.9	1.25	2.13	0.12	42.0	6.3	10.6	91.3	
DM-I-P200	38.7	2.12	3.28	0.34	29.4	7.1	7.7	88.6	
DM-II-A	31.4	1.04	1.34	0.02	47.1	5.7	6.9	93.5	85.0
DM-III-B	29.4	0.77	1.20	0	50.8	3.1	3.6	88.9	77.0
DM-III-C	35.4	0	0	0	61.5	0.65	0.3	98.9	61.8
RSD	0.8	1.6	2.9	15.9	1.0	1.4	1.4		

<sup>a</sup>K. Lig. stands for Klason Lignin.**Fig. 3.** Time-dependent cellulose conversion efficiencies of disk- and hammer-milled substrates.

easily digested by the enzymes, which contributed to the high initial hydrolysis rate. In contrast, the disk milling substrates were fractionated by a wet classifier under very dilute fiber concentrations of less than 0.2% at the start, which prevented the attachment of small fractions to the large fraction since wood fibers are hydrophilic. After completing the hydrolysis of the very small particles in certain time period, the hydrolysis of the hammer milling substrates slowed dramatically when compared to the disk milling substrates with the same specific surface, for the reason explained previously. As a result, after 24 h of enzymatic hydrolysis, the percentages of cellulose conversion of the hammer milling substrates are lower than those from the disk milling substrates (Fig. 6). This suggests that enzymes selectively attack easily accessible surfaces—the external surface of small particles in this case. The small particles not only have large specific surfaces but also may have low crystallinity and degree of polymerization from milling. The fast hydrolysis of small particles and their disappearance phenomena due to enzyme digestion agree with that reported by Schell et al. (1989).

**Fig. 4.** Comparisons of the effect of volumetric specific surface on cellulose conversion efficiency after 48 h enzymatic hydrolysis among substrates derived from various size-reduction processes.

### 5.3. Unit surface glucose yield

Fig. 7 shows the glucose yield per unit external surface after 48 h of enzymatic hydrolysis as a function of volumetric specific surface. The results indicate that DM-I produced more glucose than hammer milling, and DM-I produced more glucose than DM-II on unit-specific surfaces for the same reasons discussed in the previous section. We also observed an optimal specific surface at which unit surface glucose production reaches a maximal value for untreated DM-I substrates. The product of the  $x$  and  $y$  coordinate of Fig. 7, the unit substrate volume glucose yield (g glucose/cm<sup>3</sup> substrate), is the measure of total cellulose conversion per unit volume of wood. The total glucose yield, not the unit surface glucose yield, may be of most interest in practice. In other words, the peak unit surface glucose yield is not necessarily the economical optimum degree of size reduction. There is a trade off between size reduction and glucose yield. Increased specific surface through



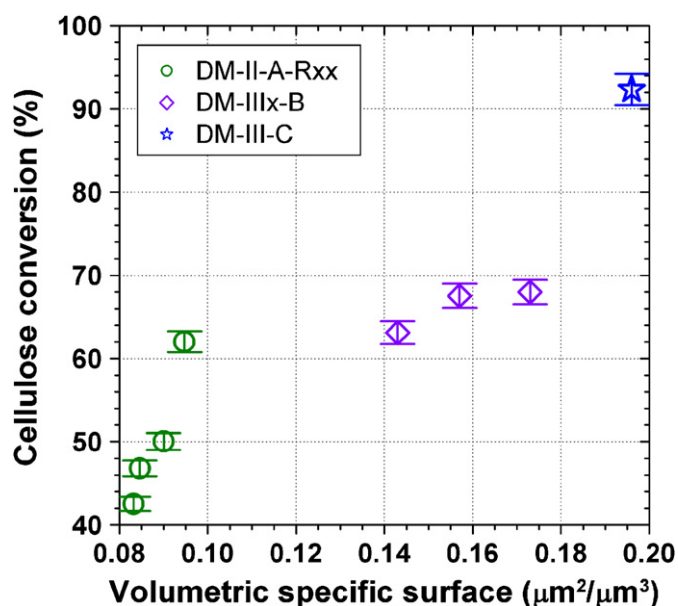


Fig. 5. Comparisons of the effect of volumetric specific surface on cellulose conversion efficiency after 48 h enzymatic hydrolysis among substrates derived from various pretreatment processes.

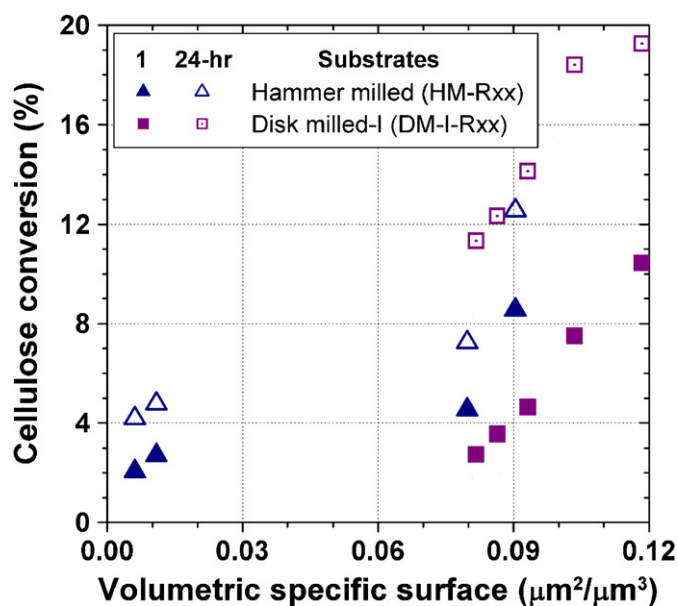


Fig. 6. Comparisons of cellulose conversion efficiency between a set of fractionated disk-milled and a set of fractionated hammer-milled substrates after 1 and 24 h enzymatic hydrolysis.

size reduction may be desirable based on total glucose yield, but the benefit of further size reduction past the peak glucose yield diminishes.

#### 5.4. Specific surface increase and size-reduction energy consumption

It is not possible to obtain electric energy consumption on the fractionated samples, as discussed previously. The hammer mill used in this study was not equipped with an energy measurement device. Therefore, the discussions in the following two sections were focused on the disk-milled whole (unfractionated) pulp samples with

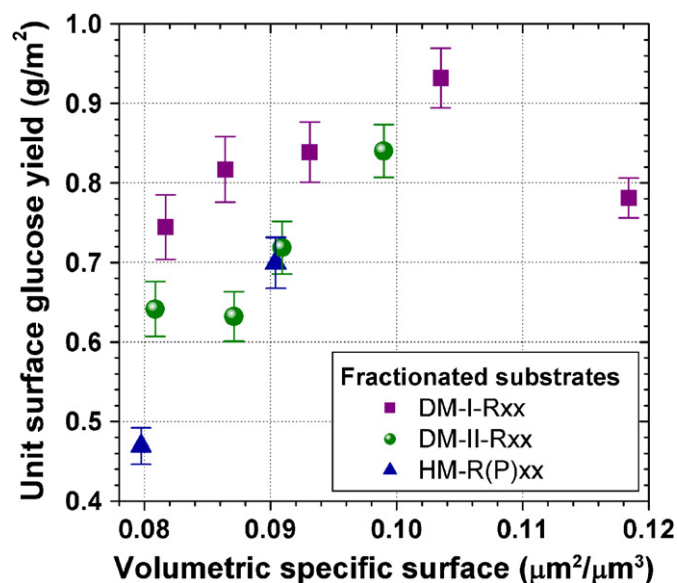


Fig. 7. Comparisons of the effect of substrate volumetric surface on unit surface glucose yield among various size-reduction processes.

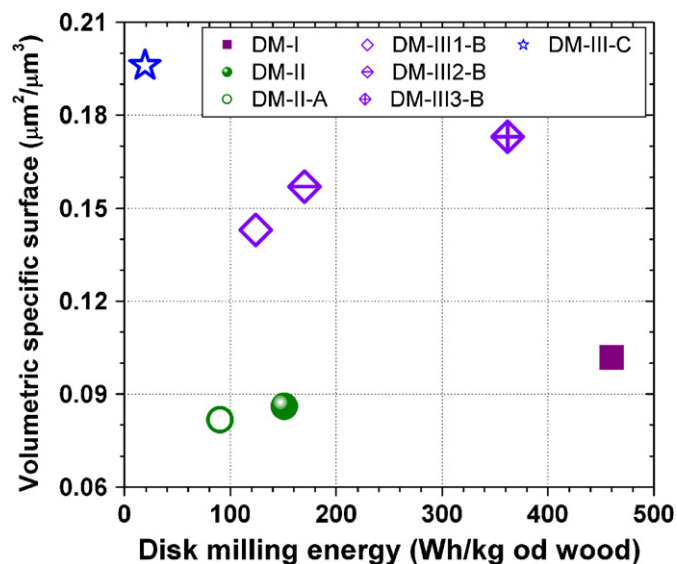


Fig. 8. Effect of chemical pretreatment on disk milling energy consumption and the resultant substrate volumetric specific surface.

the measured size-reduction energy consumption data. Fig. 8 shows the volumetric specific surface of the whole pulp samples and their corresponding electric energy consumption in producing the pulps on unit oven dry kg wood. The specific surface of the wood chips was not measured but very small with respect to the specific surfaces of the fiber samples and can be ignored. Therefore, the y coordinate can be approximated to the specific surface increase through disk milling. The energy data presented in Fig. 8 were on untreated wood mass basis, i.e., corrected for solid yield loss in the pretreatment. The data for the untreated DM-I and DM-II and DM-II-A (treated) substrates seemed to follow the same trend with disk milling energy consumption. The slope of these substrates is  $53.2 \text{ kg}/\text{m}^3 \text{ Wh}$ . The slope for the DM-III-B substrate is  $71.6 \text{ kg}/\text{m}^3 \text{ Wh}$ , greater than the untreated DM-I and DM-II substrates, indicating pretreatment (B) increased substrate surface with less energy consumption. Pretreatment C is very effective as DM-III-C falls in the very upper left corner in Fig. 8.

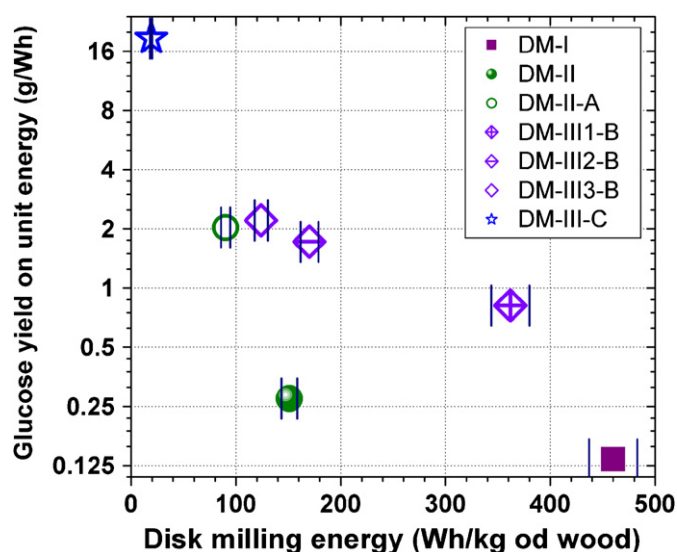


Fig. 9. Comparisons of glucose yield per unit energy consumption among various disk milling and pretreatment processes.

### 5.5. Glucose yield from unit energy consumption for size reduction

Fig. 9 shows the glucose yield per watt hour (Wh) energy consumed in mechanical milling after 48 h of enzymatic hydrolysis as a function of milling energy consumption per unit oven dry kg wood for the substrates discussed in Fig. 8. The y coordinate in Fig. 9 is a measure of size-reduction efficiency. The results indicate that DM-II is more efficient than DM-I in terms of size-reduction electric energy consumption. Using more milling energy to increase substrate surface is not effective for the pretreated substrates DM-IIIx-B. Enzymatic cellulose conversion is controlled by the physical and chemical barriers. In this case mechanical size reduction has reached the limit to further remove the physical barrier for effective enzymatic cellulose conversion. Milling conditions significantly affected the energy consumption for pretreated (B) wood chips. Furthermore, the results also indicate that pretreatment significantly improved the glucose yield per unit energy consumption. Pretreatment C is the most efficient pretreatment. It not only enhanced the enzyme accessibility to achieve cellulose conversion over 90% (Fig. 5), but it also altered the physical structure of spruce wood to significantly reduce the mechanical milling energy consumption to only 19 kJ/kg oven dry untreated wood. The product of the x and y coordinates is unit wood mass glucose yield (g glucose/kg od wood), the same measure of total cellulose conversion discussed in a previous section (Fig. 7). The economical degree of size reduction should take not only milling energy consumption but also the total glucose yield into consideration.

## 6. Conclusions

This study established a methodology for evaluating the efficiencies of mechanical (size-reduction) and chemical pretreatment processes of biomass for enzymatic saccharification. The methodology uses an integrated approach to relate substrate specific surface and energy consumption to cellulose to glucose conversion efficiency through enzymatic hydrolysis. The methodology is based on a cylinder model and a wet imaging technique that measures the two dimensions of each fiber of a substrate in a flowing channel. Imaging analysis indicated that the morphologies of biomass substrates are very different depending on the size-reduction process. Substrates derived from disk and hammer millings have a very large aspect ratio with polydispersed length and diameter/width distributions. These

substrates can be approximated as cylinders, rather than spheres. For biorefining applications, substrate surface is of most interest. The traditional sieving method that has been used almost exclusively in the published biorefining literature cannot provide the minimum necessary information to properly characterize biomass substrate.

The specific surface based on the present methodology was able to differentiate the effects of different disk milling and hammer milling processes on cellulose conversion. The fiberization process in disk milling is more effective than hammer milling, which produced fiber bundles. DM-II produced lignin-coated substrates and therefore is less effective than DM-I. These two disk millings produced higher cellulose conversion than the hammer milling. DM-I converted more cellulose than DM-II when both comparisons were made under same substrate external surfaces. However, when size-reduction energy was taken into consideration, DM-I is less effective than DM-II for cellulose conversion based on glucose yield per unit energy consumption. Chemical pretreatment can promote specific surface with less energy consumption in size reduction. Chemical pretreatment C is very efficient as it not only increased cellulose conversion but also reduce mechanical milling energy consumption significantly.

## Acknowledgments

We acknowledge the Integrated Paper Service (Appleton, WI) and Techpap (Norcross, GA) for providing complimentary characterization of substrate size using their MorFi analyzer. Tim Scott and Tom Kuster of the Forest Products Laboratory conducted imaging of some substrates. The Analytical Chemistry and Microscopy Lab of the Forest Products Laboratory conducted carbohydrate analysis.

## Appendix A.

### A.1. Mechanical size reduction

For hammer milling, spruce wood chips of moisture of about 40% were directly hammer-milled in three successive steps with decreasing screen hole size. The chips were initially processed using a semi-circular screen with 12.7 mm diameter holes (~48% open area). The accepts or undersize particles were then processed with screen hole sizes of 4.8 mm (~37% open area) and 0.8 mm (~18% open area), respectively. The material was allowed to process until the output visually ceased. For disk milling, the single disk refiner operated at 3048 rpm and used D2B-505 plate patterns. In the case of chip milling at 2.4 bar steam pressure (denoted disk milling-I, DM-I), the chips were presaturated in a separate 400 L digester in a large 45 oven dry kg batch. The digester was loaded with wood chips and was heated (purged) with steam until the temperature of the digester reached 100 °C. Then the digester was vacuumed to about 64 cmHg for over 10 min. Next, about 300 L of water at 78 °C was pulled into the digester to completely cover the chips. This water was held in the digester for 1 min and drained off. The digester was emptied, and the chips were ready for milling. The chip moisture content was increased from 40.2% to 61.3%. During milling, the 5.35 kg (od) saturated chips were allowed to preheat for 3 min at 2.4 bar steam pressure in the feed tube before processing. The disk plate gap was set at 0.254 mm. The chips were processed at about 1 kg (od)/min. The pulp was collected at the time interval of 1–5 min after the feed was started. This is the same time interval over which the electrical energy consumption was calculated. The pulp exited the refiner at 49.0% moisture. For the chips disk-milled at 7.2 bar (disk milling-II, DM-II), no presaturation treatment was applied. The chips were allowed to preheat in the feed tube for 5 min at 7.2 bar prior to feeding to the refiner. The plate gap was set at 0.06 mm. The feed rate was about 1.1 kg (od)/min. Wood chips pretreated by the sulfite

process (B and C) were milled under atmospheric conditions (disk milling-III, DM-III) with different disk gaps. Table 1 lists the disk milling experiments along with chip and milling conditions.

The electrical energy consumption of the disk refiner was collected with a digital load monitor system (Ohio Semitronics, Inc., Hilliard, Ohio, model DLM-33-480-1PR). The system displays a resettable energy consumption at the refiner and also records at 1 Hz, via RS-422, to a PC running OSI DLM software, version 1.4 (a Labview executable). The system utilizes two CTX-100-IL current transformer inputs and three voltage inputs. The manufacturer's stated accuracy is  $\pm 0.5\%$  for volts, amps, and watts and  $\pm 1\%$  for watt hours. The energy consumption (watt hours) was re-set at the local display at the beginning of each run and recorded at the end of each run. This was compared to spreadsheet-calculated energy consumption as a check. This energy consumption was arrived at by integration of the recorded power over the processing period. By spreadsheet calculation of energy consumption, it is possible to collect a sample midway through a run with an associated energy consumption over the corresponding time interval. This removes the startup and ending variability of the runs from the measurements of both fiber quality and energy consumption. Note that this starting/ending effect is small. However, it does take some time to fill the plate gap with material at the start and the feed rate does decrease slightly as the feed chamber empties. Further, small quantities of chips remain in the feed chamber, complicating the energy/mass calculation. Using either energy consumption method, the refiner operating idle energy is collected prior to a run and subtracted from the run time energy over the time period of the run. This has been the protocol for many years at our laboratory. Finally, the energy consumed is divided by the mass (od) of the fiber collected to give Wh/kg. The refiner electrical energy consumption system was calibrated by the Ohio Semitronics. No obvious drifting or data inconsistency were observed since calibration. This indirect energy determination measured the energy consumption for all the milling processes listed in Table 1.

## A.2. Biomass fractionation

For hammer milling, the resultant samples was fractionated using three screens of different mesh size into the four fractions R20, R40, R80, and P80. The sieves were from Fischer Scientific Co. (US Standard Sieve series, ASTM specifications). The screen no. 20, 40, and 80 are Tyler equivalent of 20, 35, and 80 mesh. A Ro-Tap sieve shaker (W.S. Tyler Co. Cleveland, OH) was used in the process. The device produces about 345 horizontal oscillations/min with a displacement of about 32 mm and generates about 53 hammer raps/min on top of the sieve nest. About 50 g od was used for each batch and the shaker was operated for 15 min. For disk-milled samples, the pulp was fractionated using a Bauer-McNett (TMI, Amityville, NY) wet classifier into six fractions of R14, R28, R48, R100, R200, P200 (R stands for retained on the screen, P for passing the screen). The process follows TAPPI T-233 cm-82 method. The numbers 14, 28, 48, 100, 200 represent the Tyler mesh of the screen used. The R14 fraction was further fractionated using a Pulmac shive analyzer (model MS-B2XLQ, Pulmac Instruments International, Montpelier, VT) to separate the fiber bundles (R14Rj) with a screen of slot width 0.1 mm.

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